REMARKS

A Substitute Sequence Listing is submitted herewith. The Substitute Sequence Listing shows sequences that were present in the subject application as filed and, therefore, does not add new matter.

Paragraphs at page 7, 8, 9, 48, 50, 81 and 83 have been amended as set forth in the attached "Version With Markings To Show Changes Made." It is respectfully submitted that these amendments have been made for clarification and do not add any new matter to the application. The amendment to the paragraph at page 7, 8 and 9 simply added or corrected the SEQ ID NOS listed therein. The amendment to the paragraphs at page 48 and 81 were made to correct a typographical error in the palindromic sequence in those paragraphs. The 5' G of the top sequence has been amended to a C, as would be proper in a palindromic sequence and as can be seen from the 5' C of the bottom sequence. Finally, an "I" and "R" have been added to SEQ ID NO: 39, as is shown in the figures filed with the application. As amended, the text of the application does not add any new matter.

The Examiner is invited to contact Applicants' undersigned representative if there are any questions regarding the subject application. The Commissioner is authorized to debit (or credit) Deposit Account No. 50-1355 if any fee is required (or if there is any overpayment).

CONCLUSION

If the Examiner would like to discuss any of the issues raised in this Amendment or the attached Substitute Sheets to the Sequence Listing, Applicants' representative can be reached at (858) 677-1456.

Respectfully submitted,

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EXHIBIT A

MARKED-UP COPY SHOWING AMENDMENTS TO SPECIFICATION AND CLAIM

In the Specification:

The amendment at page 7 (line 29) was as follows:

FIGURE 8 shows the amino acid sequence of the Zif268 protein (SEQ ID NO:28) and the hairpin DNA used for phage selection. (A) shows the conserved features of each zinc finger [SEQ ID NO:33 and 34] (SEQ ID NO:127, 70 and 54). (B) shows the hairpin DNA (SEQ ID NO:31) containing the 9-bp consensus binding site.

The amendment at page 8 (line 1) was as follows:

FIGURE 9 is a table listing of the six randomized residues of finger 1, 2, and 3 (SEQ ID NOS 73 through 126).

The amendment at page 8 (line 26) through page 9 (line 5) was as follows:

FIGURE 17 shows gel shift reactions. FIGURE 17A shows binding of the maltose binding protein fusions (MBP)-C7-C7 and MBP-Sp1C-C7 with duplex DNA oligonucleotides containing various target sequences. (A) MBP-C7-C7 protein was used to shift the double-stranded DNA probes containing the target sequences listed on top of each panel (from left to right; C7-C7 site (SEQ ID NO:61), Sp1C-C7 site (SEQ ID NO:62), C7 site, and $(GCG)_6$ site (SEQ ID NO:68)). The protein concentration is given in nM beneath each lane with a 2-fold serial dilution from left to right in each panel. FIGURE 17B shows MBP-SP1C-C7 protein titrated into gel shift reactions with probes containing target sequences (from left to right; Sp1C-C7 site (SEQ ID NO:62), C7-C7 site (SEQ ID NO:61), C7 site, and Sp1C site) as listed on top of each panel. The protein concentration is labeled in nM beneath each lane, with a 2-fold serial dilution from left to right in each panel.

The amendment at page 9 (line 7) was as follows:

FIGURE 18 is a DNasel footprint of MBP-C7-C7 and MBP-Sp1C-C7. A 220 bp radiolabeled fragment containing the binding site for MBP-C7-C7 (lanes 1-3) or MBP-Sp1C-

C7 (lanes 4-6) was incubated with either 20 ug/ml of BSA (lanes 2 and 4) or the cognate binding protein (300 nM, lanes 3 and 6) in 1x Binding Buffer for 30 min. DNaseI footprinting was then performed using the SureTrack Footprinting Kit (Pharmacia) according to the manufacturer's instructions. Boxed region indicates the binding site sequence (SEQ ID NOS 71 and 72). Asterisk indicates the 3'-labeled strand. Lanes 1 and 4: G+A ladders.

The amendment at page 48 (line 30) through page 49 (line 32) was as follows:

The Jun/Fos leucine zippers are described for illustrative purposes and preferentially form heterodimers and allow for the recognition of 12 to 72 base pairs. Henceforth, June/Fos refer to the leucine zipper domains of these proteins. Zinc finger Proteins are fused to Jun, and independently to Fos by methods commonly used in the art to link proteins. Following purification, the Zif-Jun and Zif-Fos constructs (SEQ ID NOS: 33, 34 and 35, 36 respectively), the proteins are mixed to spontaneously form a Zif-Jun/Zif-Fos heterodimer. Alternatively, coexpression of the genes encoding these proteins results in the formation of Zif-Jun/Zif-Fos heterodimers in *vivo*. Fusion of the heterodimer with an N-terminal nuclear localization signal allows for targeting of expression to the nucleus (Calderon, *et al, Cell*, 41:499, 1982). Activation domains may also be incorporated into one or each of the leucine zipper fusion constructs to produce activators of transcription (Sadowski, *et al., Gene*, 118:137, 1992). These dimeric constructs then allow for specific activation or repression of transcription. These heterodimeric Zif constructs are advantageous since they allow for recognition of palindromic sequence (if the fingers on both Jun and Fos recognize the same DNA/RNA sequence) or extended asymmetric sequence (if the fingers on Jun and Fos recognize different DNA/RNA sequences). For example the palindromic sequence

5 ' – [G] CGC CCA CGC $\{N\}_x$ N GCG TGG GCG – 3 '
3 ' - GCG GGT GCG CGC ACC CGC – 5 ' (SEQ ID NO:37)

is recognized by the Zif268-Fos/Zif268 Jun dimer (x is any number). The spacing between subsites is determined by the site of fusion of Zif with the Jun or Fos zipper domains and the length of the linker between the Zif and zipper domains. Subsite spacing is determined by a

binding site selection method as is common to those skilled in the art (Thiese, et al., *Nucleic Acids Research*, 18:3203, 1990). Example of the recognition of an extended asymmetric sequence is shown by Zif(C7)$_6$-Jun/Zif-268-Fos dimer. This protein consists of 6 fingers of the C7 type (EXAMPLE 11) linked to Jun and three fingers of Zif268 linked to Fos, and recognizes the extended sequence:

5 ' – CGC CGC CGC CGC CGC CGC $\{N\}_X$ N GCG TGG GCG – 3 '
3 ' – GCG GCG GCG GCG GCG GCG CGC ACC CGC – 5 '
(SEQ ID NO: 38)

The amendment at page 50 (line 29) through page 51 (line 15) was as follows:

Following mutagenesis and selection of variants of the Zif268 protein in which the finger 1 specificity or affinity is modified, proteins carrying multiple copies of the finger may be constructed using the TGEKP (SEQ ID NO:67) linker sequence by methods known in the art. For example, the C7 finger may be constructed according to the scheme:

MKLLEPYACP VESCDRRFSK SADLKRHIR(I)HTGEKP-
(YACPVESCDRRFSKSADLK(R)HIRIHTGEKP)$_{1\text{-}11}$, (SEQ ID NO:39) where the sequence of the last linker is subject to change since it is at the terminus and not involved in linking two fingers together. This protein binds the designed target sequence GCG-GCG-GCG [(SEQ ID NO:32)] in the oligonucleotide hairpin CCT-CGC-CGC-CGC-GGG-TTT-TCC-CGC-GCC-CCC GAG G (SEQ ID NO:40) with an affinity of 9nM, as compared to an affinity of 300 nM for an oligonucleotide encoding the GCG-TGG-GCG sequence (as determined by surface plasmon resonance studies). Fingers utilized need not be identical and may be mixed and matched to produce proteins which recognize a desired target sequence. These may also be utilized with leucine zippers (*e.g.*, Fos/Jun) or other heterodimers to produce proteins with extended sequence recognition.

The amendment at page 81 (line 27) through page 82 (line 26) was as follows:

The Jun/Fos leucine zippers preferentially form heterodimers and allow for the recognition of 12 to 72 base pairs. Henceforth, Jun/Fos refer to the leucine zipper domains of

these proteins. Zinc finger proteins are fused to Jun, and independently to Fos by methods commonly used in the art to link proteins. Following purification, the Zif-Jun and Zif-Fos constructs (FIGURE 13 and 14, respectively), the proteins are mixed to spontaneously form a Zif-Jun/Zif-Fos heterodimer. Alternatively, coexpression of the genes encoding these proteins results in the formation of Zif-Jun/Zif-Fos heterodimers *in vivo*. Fusion with an N-terminal nuclear location signal allows for targeting of expression to the nucleus (Calderon, *et al., Cell*, 41:499, 1982). Activation domains may also be incorporated into one or each of the leucine zipper fusion constructs to produce activators of transcription (Sadowski, *et al., Gene*, 118:137, 1992). These dimeric constructs then allow for specific activation or repression of transcription. These heterodimeric Zif constructs are advantageous since they allow for recognition of palindromic sequences (if the fingers on both Jun and Fos recognize the same DNA/RNA sequence) or extended asymmetric sequences (if the fingers on Jun and Fos recognize different DNA/RNA sequences). For example the palindromic sequence

5 ' – [G] CGC CCA CGC $\{N\}_x$ N GCG TGG GCG – 3 '
3 ' - GCG GGT GCG CGC ACC CGC – 5 ' (SEQ ID NO:37)

Is recognized by the Zif268-Fos/Zif268 Jun dimer (x is any number). The spacing between subsites is determind by the site of fusion of Zif with the Jun or Fos zipper domains and the length of the linker between the Zif and zipper domains. Subsite spacing is determined by a binding site selection method as is common to those skilled in the art (Thiese, *et al., Nucleic Acids Research*, 18:3203, 1990). Example of the recognition of an extended asymmetric sequence is shown by Zif(C7) $_6$-Jun/Zif- 268-Fos dimer. This protein consists of 6 fingers of the C7 type (EXAMPLE 11) linked to Jun and three fingers of Zif268 linked to Fos, and recognizes the extended sequence:

5 ' – CGC CGC CGC CGC CGC CGC $\{N\}_x$ N GCG TGG GCG – 3 '
3 ' – GCG GCG GCG GCG GCG GCG CGC ACC CGC – 5 '
(SEQ ID NO: 38)

The amendment at page 83 (line 5) was as follows:

Following mutagenesis and selection of variants of the Zif268 protein in which the finger 1 specificity or affinity was modified (See EXAMPLE 7), proteins carrying multiple copies of the finger may be constructed using the TGEKP (SEQ ID NO:67) linker sequence by methods known in the art. For example, the C7 finger may be constructed according to the scheme:

MKLLEPYACP VESCDRRFSK SADLKRHIR(I)HTGEKP-(YACPVESCDRRFSKSADLK(R)HIRIHTGEKP)$_{1-11}$, (SEQ ID NO:39), where the sequence of the last linker is subject to change since it is at the terminus and not involved in linking two fingers together. An example of a three finger C7 construction is shown in Figure 15. This protein binds the designed target sequence GCG-GCG-GCG [(SEQ ID NO:32)] in the oligonucleotide hairpin CCT-CGC-CGC-CGC-GGG-TTT-TCC-CGC-GCC-CCC GAG G (SEQ ID NO:40) with an affinity of 9nM, as compared to an affinity of 300 mM for an oligonucleotide encoding the GCG-TGG-GCG sequence (as determined by surface plasmon resonance studies). Proteins containing 2 to 12 copies of the C7 finger have been constructed and shown to have specificity for their predicted targets as determined by ELISA (see for example, EXAMPLE 7). Fingers utilized need not be identical and may be mixed and matched to produce proteins which recognized a desired target sequence. These may also be utilized with leucine zippers (*e.g.*, Fos/Jun) to produce proteins with extended sequence recognition.